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Pulmonary and Systemic Health Effects of Acute and Subchronic Exposure to Smoke Obscurant SGF-2

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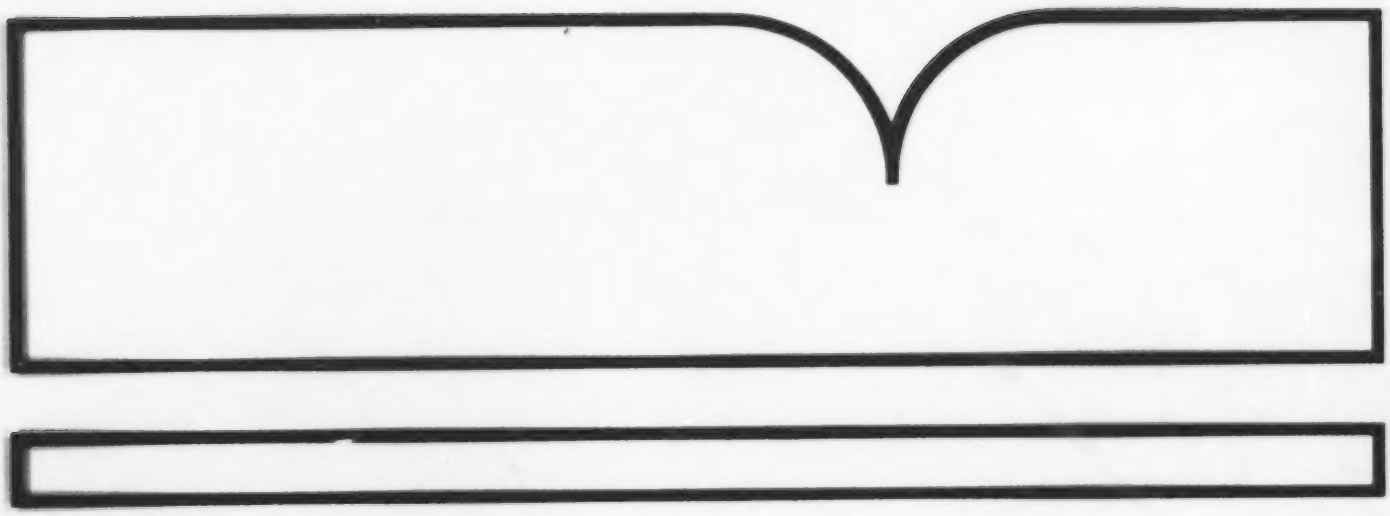

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PULMONARY AND SYSTEMIC HEALTH EFFECTS OF ACUTE AND SUBCHRONIC EXPOSURE TO SMOKE OBSCURANT SGF-2

by

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16. ABSTRACT

Sixty-day old, male rats were exposed to air, 0.5 or 1.5 ml/l fog-oil for 3.5 hr/d, 4 days/wk for either 4 or 13 wk. Following the acute (4 wk) exposure to 1.5 mg/l, a multifocal pneumonitis was observed. Lung lavage fluid had an elevated number of polymorphonuclear leukocytes, alveolar macrophages, total cells and an increase in lavage fluid protein. Lung wet and dry weights were increased. A lesser increase in the number of alveolar macrophages and lung weights was observed after exposure to 0.5 mg/l. Pulmonary function tests revealed an increase in end expiratory volume (EEV) in animals exposed to 1.5 mg/l. A decrease in zoxazolamine-induced paralysis time was observed following both 0.5 and 1.5 mg/l, however, no effect on pentobarbital-induced sleep time was observed. The subchronic (13 wk) exposures resulted in decreased body weight and increased lung dry weights at both 0.5 and 1.5 mg/l, an increase in lavage fluid protein and EEV following exposure to the 1.5 mg/l concentration. Zoxazolamine paralysis time, which was decreased after both concentrations, correlated with an increase in aryl hydroxylhydrolase (AHH) activity in the liver. In conclusion, it appears that inhalation of smoke SGF-2 causes minimal pulmonary and systemic effects.

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ABSTRACT

Sixty-day old, male Sprague-Dawley rats were exposed to 0.5 or 1.5 mg/l fog-oil for 3.5 hr/d, 4 days/wk for either 4 or 13 wk. Control rats were subjected to similar conditions while breathing filtered air. Both pulmonary and systemic effects were investigated one day after the exposure ceased. For the 13 wk exposure groups, animals were also examined 30 days after the exposure. Following the acute (4 wk) exposure to 1.5 mg/l, a multifocal pneumonitis was observed. Lung lavage fluid had an elevated number of polymorphonuclear leukocytes, alveolar macrophages, total cells and an increase in lavage fluid protein. Lung wet and dry weights were increased. A lesser increase in the number of alveolar macrophages and lung weights was observed after exposure to 0.5 mg/l. Pulmonary function tests revealed an increase in end expiratory volume (EEV) in animals exposed to 1.5 mg/l. Systemic effects after the acute exposure were minimal. A decrease in zoxazolamine-induced paralysis time was observed following both 0.5 and 1.5 mg/l, however, no effect on pentobarbital-induced sleep time was observed. Thus, it appears that cytochrome P-448-related metabolism is more affected than P-450 metabolism. A dose-related decrease in mean corpuscular volume and mean corpuscular hemoglobin correlated with an increase in RBC's, but no other observable hematological changes occurred. Behavioral studies were negative, as were clinical chemistries and immune function tests. The subchronic (13 wk) exposures resulted in decreased body weight and increased lung dry weights at both 0.5 and 1.5 mg/l. As in the 4 wk study, there was an increase in lavage fluid protein and EEV following exposure to P-448 the 1.5 mg/l concentration. Zoxazolamine paralysis time, which was decreased after both concentrations, correlated with an increase in aryl hydroxylhydrolase (AHH) activity in the liver. There were no observable changes in immunological parameters or clinical chemistries. In conclusion, it appears that inhalation of smoke SGF-2 causes minimal pulmonary effects. Systemically, there was an induction of cytochrome, which could have significant implications for xenobiotic metabolism.

1. INTRODUCTION

Chemical smokes/obscurants are used by the military to conceal personnel, materiel, and installations from direct visual observation. The smoke from a petroleum distillate product is generated by injecting a light lubricating oil into a heated engine exhaust manifold where it vaporizes and eventually recondenses in the atmosphere. Army personnel may be exposed to this chemical smoke when it is released into the environment in training or combat operations. Since this petroleum smoke is a large area screening obscurant, the duration of exposure is usually hours within a single day, and exposures may be repeated over consecutive days. Evaluation of the potential hazards posed by this smoke to human health is a necessary portion of the data base required to establish comprehensive health criteria for the field use of smokes and obscurants. The purpose of this study was to assess biologic effects of short-term (4 wk) and subchronic (13 wk) inhalation exposures of fog-oil.

2. METHODS

2.1 ANIMALS

Male and female CD rats (Charles River Breeding Laboratories, Kingston, N. Y.) were used throughout this study, except for immunological parameters which require inbred (Fischer 344) rats (same sex and source). Rats were received at approximately 60 days of age, and quarantined for at least 4 days. At the time exposures began, animals were 64-72 days old and weighed approximately 300 g. All animals were anesthetized with sodium pentobarbital at time of sacrifice.

2.2 EXPOSURE CONDITIONS

All exposures were carried out according to methods described in the documentation for the inhalation exposure facility (Davies, 1984). Animals received a whole-body inhalation exposure in Rochester-type chambers. The oil was injected onto a Vycor glass heating element in an inert nitrogen atmosphere. As the oil vapor/nitrogen mixture flowed through the generator, the temperature was maintained by a heat tape to prevent premature condensation. The mixture was injected into the generator portion of the dual path chamber inlet air duct. The oil vapor quickly condensed to form the smoke when diluted with chamber supply air. The aerosol passed through an orifice plate used to monitor flow and to enhance stream mixing. The aerosol entered at the top of the chamber and was deflected to the chamber perimeter by a conical baffle. Two perforated plates at the top of the chamber promoted mixing and uniform aerosol distribution. Average droplet size of the aerosol, measured by a 7-stage cascade impactor, was approximately 1 to 1.3 μm mass median aerodynamic diameter (MMAD) with a geometric standard deviation of 1.5. Chamber aerosol concentrations were monitored using Real-time Aerosol Monitors (RAM-1). These sensors were calibrated to gravimetric filter samples. A minimum of two quality control filter samples per exposure, per chamber were collected and weighed to test the accuracy of electronic concentration monitors and archived for possible later use.

2.3 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES

2.3.1 Exposure Regimens

For most of the biologic parameters examined, in the 4 week studies, male rats were exposed 3.5 hrs/d, 4 d/wk, to either 0 (air), 0.5, or 1.5 mg/l concentrations of fog oil, for 4 weeks. Since two replications of this regimen were available, the statistical analysis model utilized replication, fog oil concentration, and the interaction between these two factors. Edema was examined in both male and female rats exposed for 4 weeks. In this instance, the design employed was a 3x2 factorial design with fog oil concentration at 3 levels (0, 0.5, and 1.5 mg/l) for each sex. The concentrations of fog oil chosen were based upon range finding studies in which no excess mortality was observed for this length and duration of exposure.

In the 13 week exposure studies, rats were exposed 3.5 hrs/ day, 4 days/week to one of 3 exposure concentrations (0.0, 0.5 and 1.5 mg/l). Animals in each exposure group were used to evaluate biologic parameters on the day after the last exposure. This experimental regimen corresponded to a one-way analysis of variance with fog oil concentration at 3 levels.

2.3.2 Statistical Techniques

Basically the same analytical strategies were employed in both the 4 and 13 week analyses. For endpoints in which more than one biologic parameter was being measured, both univariate and multivariate analysis of variance (ANOVA) models were fit to the data. Preliminary analysis of each endpoint examined for homogeneity of variance and normality of residuals. In the event that either of these ANOVA assumptions was strongly violated, analysis of a mathematically transformed variable or analysis by nonparametric methods was be considered. When the appropriate analysis was a 2 factor ANOVA, main effects were examined for statistical significance only when there was no evidence of a significant interaction.

2.4 BIOLOGICAL PARAMETERS (Table 1)

2.4.1 Pulmonary Physiology (anesthetized animals).

The animals were anesthetized, tracheostimized, and the following measurements performed: lung volumes, quasi-static compliance, diffusing capacity, distribution of ventilation (N2 washout), wet and dry lung weights (Raub et al., 1982; Takezawa et al., 1980).

2.4.2 Immunology

Fischer 344 rats were anesthetized, bled out from the aorta, and their spleens removed. Spleens and peripheral blood from individual rats were processed, and the response of cells to phytohemagglutinin and

concanavalin A were assessed (Selgrade, et al., 1976). The natural killer cell activity of the same spleen cell preparations was also assessed (Selgrade, et al., 1982).

2.4.3 Clinical Chemistry

Following exposure, blood samples were collected from the dorsal aorta immediately after cervical dislocation. In order to evaluate potential target organ toxicity, the following clinical chemistry assays were performed according to standard procedures (Baker Instruments, Calbiochem-Behring and Boehringer Mannheim): albumin, aldolase, alkaline phosphatase, amylase, total bilirubin, blood urea nitrogen, calcium, cholinesterase, cholesterol, creatine kinase, creatinine, glucose, lactate dehydrogenase, leucine aminopeptidase, inorganic phosphate, total protein, SGOT, SGPT, triglyceride, and uric acid. The lactate dehydrogenase and triglyceride assays were performed on the same day the blood samples were collected. The remaining serum samples were stored at -80°C until analysis (Young et al., 1975).

2.4.4 Histopathology

All tissues were examined in situ, dissected from the carcass, trimmed, weighed and reexamined before fixation. Tissue samples for fixation were < 0.5 cm in thickness. Lungs were fixed in their entirety after being reinflated with formalin fixative under 25-30 cm water pressure. The calvarium was removed and the brain freed and weighed. Then the entire skull, including the nasal cavity (turbinates) and pituitary, was placed in fixative.

The following tissues were sectioned and stained with hematoxylin and eosin using routine histopathologic procedures: gross lesions, duodenum, liver, testes/epididymis, lungs (two sections - right diaphragmatic and left lobe), nasal cavity and nasal turbinates (three separate sections), heart, stomach, larynx, trachea (distal), kidneys, eyes, skin (mid-dorsal region), and peribronchial lymph nodes.

2.4.5 Hematology

Animals were anesthetized and bled from the aorta. Blood was preserved with heparin and complete blood count (CBC) and leukocyte differential tests were performed.

2.4.6 Pulmonary Edema

Lungs were lavaged according to the method of Hatch et al. (1985). Samples of lavage fluid were then stored frozen (-20°C) until assayed for total protein content.

2.4.7 Pulmonary Cell Populations

After animals were anesthetized, they were intratracheally injected with Streptococcus sp. (Group C) and the lungs lavaged 30 min later to measure phagocytosis. Total and differential cell counts and cell viability were obtained at the same time (Gardner et al., 1977; Coffin et al., 1968).

2.4.8 Xenobiotic Metabolism

To determine whether the obscurant affected xenobiotic metabolism, pentobarbital-induced sleep time (ST) and zoxazolamine paralysis time (PT) were investigated. Rats were given intramuscular injections of 25 mg/kg sodium pentobarbital. Time to loss of righting reflex and sleep time were assayed (Graham, et al., 1982). A separate group of animals were injected with 100 mg/kg zoxazolamine and the induction time and paralysis time assayed (Graham, et al., 1982).

Cytochrome P-450 levels and aryl hydrocarbon hydroxylase (AHH) activity were also investigated. Livers from individual rats were homogenized and the microsomal fraction obtained. The sample was then divided into two aliquots - one for the cytochrome P-450 assay and one for the AHH activity. Cytochrome P-450 levels were determined according to the methods of Omura and Sato (1964). The AHH assay was carried out according to the methods of DePierre et al. (1975).

2.4.9 Behavioral Response

Procedures for determination of rat activity in a figure-eight maze are detailed elsewhere (Reiter, et al., 1975). The activity of rats in the maze was recorded via eight photodiode pairs mounted in the maze.

2.4.10 Cardiopulmonary Physiology

Rats were anesthetized and surgically implanted with an intrapleural and a carotid arterial catheter. Data were collected on pulmonary mechanics, EKG, and blood gases. Procedures for the rats were the same as those described for guinea pigs (Grose, et al., 1984).

2.4.11 Body Weight

All animals were weighed before the first exposure and after the last exposure. In addition, prior to exposure 10 animals per treatment group were selected at random from among those to be used for various other parameters. These animals were weighed before the first exposure of each week and after the last exposure of each week.

TABLE 1. BIOLOGICAL PARAMETERS ASSAYED

Parameter	4 week study	13 week study
Aryl Hydrocarbon Hydroxylase		X
Behavior	X	
Body Weight	X	X
Cardiopulmonary Physiology	X	
Clinical Chemistry	X	X
Cytochrome P-450		X
Edema (M,F)*	M and F	M
Hematology	X	
Immunology	X	X
Lung Cell Differentials	X	
Paralysis Time - Zoxazolamine	X	X
Pathology	X	X
Phagocytosis	X	
Pulmonary Physiology	X	X
Sleep time - Pentobarbital	X	

*M=Male; F=Female

3. RESULTS AND DISCUSSION

3.1 4 WEEK STUDY

3.1.1 Pulmonary Physiology

Eleven endpoints were selected to assess the effects of fog oil on pulmonary physiology in anesthetized animals. Ending expiratory volume (EEV) ($p = 0.006$), dry lung weight ($p = 0.0001$), and wet lung weight ($p = .0002$) were the only responses that showed significant dose-related effects pooled over both replicates. Individual contrasts of mean control versus mean response at each of the two fog oil doses indicated that for EEV, the only significant difference occurred between controls and 1.5 mg/l ($p = 0.002$). The same situation occurred for dry and wet lung weights ($p = 0.0001$). From these results, we conclude that exposure of CD rats to 1.5 mg/l fog oil, 3.5 hr/d, 4 d/wk for 4 weeks results in a significant increase in lung wet and dry weight and in end expiratory volume. None of the other pulmonary physiological parameters showed significant effects. None of the 11 parameters tested showed significant effects at the 0.5 mg/l concentration. Because both wet and dry weight were affected at the 1.5 mg/l level in the same manner, it appears that there was an increase in lung cellularity. This could be attributable to a variety of causes. Since diffusing capacity was unchanged and normal gas exchange was maintained, we speculate that the significant increase in EEV at 1.5 mg/l may be an attempt to compensate for some "change" in the alveoli. Oil deposition or cellular infiltration are two possible causes of such a change.

3.1.2 Immunology

Two types of immune functions were examined: a) natural killer cell (NK) activity in spleen cells and (b) response of spleen and peripheral blood, white cells to concanavalin A and phytohemagglutinin (ConA and PHA; T cell mitogens) and to pokeweed mitogen (PWM; T and B cell mitogen).

NK activity was determined at 4 effector to target cell ratios ranging from 1 to 25 to 1 to 200 and increasing by 2-fold intervals. The multivariate analysis showed only an effect due to concentration ($p = 0.05$), that was due to an increase in cytotoxicity in the fog oil exposed groups. Qualitatively there appears to be some increase in NK activity in fog oil-exposed animals exposed 4 d/wk for 3.5 hr. However, post hoc t-tests showed that the only results approaching significance were at the 1:50 ratio. For the 1:50 assay, the difference between control and the 1.5 mg/l group mean for 4/wk, 3.5 hr a day yielded a significance of 0.03. We conclude that fog oil causes some stimulation of NK activity, suggesting that the immune system is being exposed to foreign antigens. There was no effect of fog oil concentration on splenic or peripheral lymphocytes togens. If there are effects on mitogen-induced blastogenesis of lymphocytes to these fog oil doses we did not have enough power to detect them.

3.1.3 Clinical Chemistry

Analysis of the clinical chemistry parameters showed no statistically significant changes due to treatment. From a qualitative standpoint, examination of the means, pooled over replicates, revealed a monotonic increase or decrease, with respect to fog oil dose, for albumin, cholesterol, bilirubin, cholinesterase, leucine-aminopeptidase, inorganic phosphorous and protein. Since there is a 33% chance of seeing a monotonic increase or decrease in the examination of mean responses at 3 doses, and since we examined 20 variables, we could expect 6 or 7 variables to show such a trend by chance. We therefore conclude that the test conditions chosen cause no substantial effects on clinical chemistries.

3.1.4 Histopathology

No significant microscopic findings were observed in the nasal cavity, eyes, heart, skin, larynx, trachea, kidney, liver, stomach, duodenum, testes and epididymides. However, a few incidental findings such as focal chronic myocarditis, focal tubular regeneration in the kidney, unilateral seminiferous tubular atrophy and a corresponding lack of sperms in the epididymis were observed in an occasional rat. Focal hemorrhage, histiocytosis, and focal hemosiderosis were observed in an occasional peribronchial lymph node from all dose groups.

Histopathologic findings were similar in the right diaphragmatic and left lobes of the lung. A minimal amount of peribronchial lymphocytic infiltrate was present in most lungs and was considered to be normal. A moderate number of macrophages were scattered throughout the pulmonary alveolar lumens of most animals treated for 3.5 hours. Usually only a few macrophages were in an alveolus and these alveolar macrophages contained eosinophilic (protein-like) material in their cytoplasm. The number of macrophages in the alveoli was greater in the highest dose group exposed for the longest length of time (3.5 hours per day for four days per week at 1.5 mg/l). Thus, it appears that a degree of inflammation was present.

3.1.5 Hematology

The eight hematology parameters measured were analyzed by MANOVA model. There was no indication of a multivariate interaction ($p = 0.64$, Wilks' criterion) or an overall fog oil concentration effect ($p = 0.18$, Wilks' criterion).

The univariate ANOVA for mean corpuscular volume indicated a highly significant main effects test ($p = .0054$) due to exposure concentration. Changes in the number of RBC were marginally significant, given the number of responses examined. Thus, while the volume of RBC's was significantly decreased with increasing concentrations of fog oil ($p < 0.05$ at the 1.5 mg/l level), the number of red blood cells appeared to increase with fog oil concentration.

3.1.6 Pulmonary Edema

The ANOVA of male rat responses indicated a significant elevation of lavage fluid protein (LFP). Subtesting showed an effect at the 1.5 mg/l exposure level ($p = 0.0023$) but not at the lower level of exposure. This suggests that edema occurred at this dose and is consistent with the influx of polymorphonuclear leukocytes which was observed.

To examine possible sex differences in susceptibility to edema, male and female rat responses were compared in a separate experiment. A two-way ANOVA showed no interaction between sex and fog oil concentration on LFP, indicating that the response of males and females was statistically indistinguishable. The LFP for males were not significantly higher at the 1.5 level. However, the increase seen in females was significant, and when data from males and females were pooled, the increase in LFP was significant.

3.1.7 Pulmonary Cell Populations

The ANOVA indicated a significant increase in total cells following exposure to 1.5 mg/l, which appeared to be due to a significant ($p = 0.0001$) influx of polymorphonuclear leukocytes (PMN) indicative of an inflammatory response. The analysis also indicated a significant ($p = 0.030$) increase in total cells at 0.5 mg/l, but percent PMN was not significantly increased at that concentration. Viability of the pulmonary cells appeared to be significantly enhanced by the fog oil treatment, at both the 0.5 mg/l and 1.5 mg/l concentrations. Effects were actually greater at 0.5 than at 1.5 mg/l. The mechanisms responsible are unknown. However, it is possible that fog oil caused a lysis of resident non-viable cells and/or the influx of new cells, primarily PMN, had a higher viability. Reproducible changes in alveolar macrophage and lymphocyte populations were not noted.

Analysis of percent phagocytosis indicated no difference between control and exposed groups. Thus we can conclude from this data that fog oil exposure causes an influx of inflammatory cells, but does not adversely affect phagocytic function.

3.1.8 Xenobiotic Metabolism

Pentobarbital metabolism is more related to cytochrome P-450 activity while zoxazolamine metabolism is more related to P-448. Other differences in the nature of metabolism also exist between these two drugs.

The two parameters measured by the pentobarbital assay, time to loss of righting reflex after injection of sodium pentobarbital and elapsed sleeping time, were examined using both multivariate and univariate ANOVA methods. Animals exposed 4 times/wk showed decreasing mean number of min. to loss of righting reflex with increasing fog oil dose. Using post hoc t-tests of the least square means, the response at 0.5 mg/l was 10.6 min., marginally ($p = 0.086$) lower than the control response of 12.4 min. The mean response at 1.5 mg/l, 9.4 minutes, was

markedly different ($p = 0.006$) from the control response. With respect to pentobarbital-induced sleeping time, we observed a significant ($p = 0.02$) effect due to fog oil dose. A significant decrease in pentobarbital-induced sleeping time is typically indicative of induction of the cytochrome P-450 system.

As in the pentobarbital study, two time measurements were taken after zoxazolamine treatment; the time from injection to loss of righting reflex and the time the animal remained paralyzed. Logarithms of both measures were taken to improve compliance with the assumptions of homogeneity of variance and normality of residuals. The univariate ANOVA of paralysis time indicated highly significant effects due to exposure concentration of fog oil ($p = 0.0001$). Least squares geometric means were 228, 108, and 87 min. for the air, 0.5 mg/l, and 1.5 mg/l exposure groups, respectively. These data tend to indicate that the cytochrome P-448 may be induced by the fog oil exposure. It appears that cytochrome P-448-related metabolism may be affected more than cytochrome P-450-related metabolism, an effect consistent with the organic content of the fog oil.

3.1.9 Behavioral studies

Activity of rats in a figure-8 maze was recorded via eight photodiode pairs mounted in the maze. The two-way ANOVA for animals exposed four times per week did not detect any significant interaction or main effect. Given the lack of a meaningful biological explanation for the observed behavioral patterns, it appears most likely that the effects of the 4-week exposures on this parameter were either negligible or not detectable with these sample sizes. Using the methodology of Cohen (1977), the power of detecting a significant effect in this behavioral activity at the 5% significance level due to fog oil concentration, given the effect size seen here and the sample size per treatment group, is less than 20%. To achieve 85% power with this effect size, nearly 50 animals/concentration would be required when testing at this significance level. Tests requiring such large numbers of animals were not feasible.

3.1.10 Cardiopulmonary Physiology

The data from five control and five treated animals revealed no significant differences in pulmonary function. Although not statistically significant, the data suggested increased expiratory resistance in treated animals which may have caused a slight elevation in breathing rate and intrapleural pressure. Because only three treated and three control rats were examined for cardiovascular effects, results are inconclusive.

3.1.11 Body Weight

Two types of data were collected: (a) the weights of one subset of 6 animals/group were measured immediately before the first and after the last exposure for each of the 4 weeks; (b) weights of all the animals were routinely measured before the first and after the final exposure in the study.

During the first week, although weight gain decreased with increasing fog oil concentration, there was a much greater difference between the air group and the 1.5 mg/l group (22.3 gm). For the remaining 3 weeks, weight gains even in the 0.5 mg/l group were significantly lower than in controls. In all cases, weight gain decreased as concentration of fog oil increased. We conclude from these studies that increases in concentration of fog oil causes decreases in weight gain during the weekly exposures. This appears to be a result of fog oil exposed animals not eating during the days when exposures were occurring. During the intervening days from one weekly exposure to the next, the animals did eat and made up for some of the weight lost during the exposures. The total weight change from just prior to the first exposure until immediately after the last exposure was examined in all animals. Animals exposed to air for 4 weeks gained an average of 100 g, rats exposed to 0.5 mg/l fog oil gained 87 g, and those exposed to 1.5 mg/l gained 78 g.

Given these results, it is again reasonable to conclude that increasing fog oil concentration contributed to a lowering of weight gain over the entire 4 week course of exposure. Increasing the frequency of exposure did not seem to affect weight gain. All animals weighed about 300g when exposures began, and control animals weighed about 400g by the end of the 4 week exposure period. Even in the worst case, the weight difference between fog oil and control animals was less than 10%. This difference appears to be due to an anorexic response on the days when exposures occurred and does not appear to represent gross toxicity. Since the difference is small and can be adjusted for, it does not pose a problem in the interpretation of data for other parameters. Table 2 summarizes the results of the 4 week study.

TABLE 2. SUMMARY OF 4 WK DATA

Parameter	Effect
Histopathology	Dose related ↑ in alveolar macrophages. Pneumonitis in 4/6 males exposed at 1.5 mg/l.
Lavage Fluid Protein	↑ at 1.5 mg.l; no male/female difference.
Lavage Fluid Cells	↑ in PMN's and total cells at 1.5 mg/l.
Pulmonary Function	↑ EEV, lung wet and dry weight.
Zoxazolamine-Induced Paralysis Time	Decreased at 0.5 and 1.5 mg/l.
Pentobarbital-induced Sleep Time	Probably decreased at 1.5 mg/l.
Hematology	Dose related decrease in mean corpuscular volume and mean corpuscular hemaglobin, increase in RBC's, no change in hemaglobin and other parameters.
Clinical Chemistry	No significant effects
Immunology	Possible enhancement of N.K. No other effects.
Behavioral	No effects.

3.2 SUBCHRONIC (13 WEEK) STUDY

3.2.1 Pulmonary Physiology

The multivariate test of no overall dose effect for the pulmonary physiology parameters viewed jointly resulted in a significance probability of 0.0005 using Wilk's criterion. Univariate analyses of each parameter showed that dose-related changes in end expiratory volume ($p = 0.042$), wet lung weight ($p = 0.0004$), dry lung weight ($p = 0.0001$), and body weight ($p = 0.0165$) contributed most to the overall effect. These were the same parameters which showed significant effects in the 4-week study. Williams tests on these parameters indicated that for dry lung weights, the means at 0.5 mg/l were significantly greater than their respective controls. For wet lung weight, a significant increase was seen only at the 1.5 mg/l level. No difference between any dose group and control response could be seen for end expiratory volume (EEV) using a 2-sided Williams test. However, using a less conservative subtest (a t test of least square mean values), this difference was significant ($p = 0.028$). It is not uncommon to observe increases in EEV when animals are exposed to an irritant. The animal may simply be responding to the exposure by taking more shallow breaths. The effects on lung weights may indicate an increase in lung cellularity. Results from histopathology (Section 3.2.4) are helpful in the interpretation of these data.

3.2.2 Immunology

Fog oil exposure had no significant effects on the response of lymphocytes from spleen and peripheral blood to 3 different mitogens -phytohemagglutinin (PHA), concanavalin A (ConA), and pokeweed mitogen (PWM). Although there were some concentration-dependent trends, the spleen and peripheral blood responses, with the exception of ConA, were not consistent.

Square roots of the differential counts were analyzed for both spleen and peripheral blood mitogen responses. Six responses were analyzed simultaneously in a one-way MANOVA. This joint response did not appear to be affected ($p = 0.67$, Wilk's criterion) by fog oil concentration. None of the univariate ANOVA's showed indications of a concentration-related effect. Significance probabilities ranged from $p = 0.11$ to 0.76. We conclude from these data that the ability of lymphocytes to mount a blastogenic response when stimulated is not impaired by fog oil exposure. Fog oil exposure also had no effect on natural killer cell activity (an important mediator of immune surveillance) when tested at 4 effector to target cell ratios.

3.2.3 Clinical Chemistry

As in the 4-wk study, none of these parameters were significantly affected by fog oil exposure. The one exception, triglycerides, showed a significant decrease at the 1.5 mg/l level. Six other parameters (total protein, triglycerides, leucine aminopeptidase, creatine kinase,

cholesterol and calcium) did show a qualitative concentration-dependent effect due to fog oil exposure. As pointed out above, there is a 33% chance of seeing a monotonic increase or decrease in the examination of mean responses at 3 concentrations. Since we are examining 20 variables, we would expect 6 or 7 variables to show such a trend by chance. A multivariate ANOVA by fog oil dose did not show a significant joint response ($p = 0.66$, Wilk's criterion). Of the 20 univariate ANOVA's, only triglycerides ($p = 0.07$) showed any evidence of a concentration-related effect. In view of the number of parameters tested, even this marginal effect should be viewed with caution. At an α-level of 7%, we would expect 1 or 2 tests to be significant if the tests were independent. Williams test indicated that the decrease in triglyceride levels was significant at the 1.5 mg/l level. In any case, an increase in triglycerides is the effect more likely to be associated with health effects. We conclude from these studies that clinical chemistry parameters are not affected in a meaningful way by fog oil exposures.

3.2.4 Histopathology

Treatment-related microscopic changes were observed in the lungs and the peribronchial lymph nodes of male rats following 13 wk of exposure to fog oil. A diffuse accumulation of macrophages were present in the pulmonary alveoli. Although the distribution of macrophages involved all regions of the pulmonary parenchyma, there was an increased number of alveolar macrophages in the alveoli near the terminal bronchioles. The degree of severity was concentration-related with moderate to moderately severe involvement in the 1.5 mg/l exposure group and minimal to slight involvement in the 0.5 mg/l exposure group. Other changes observed in the lungs which appeared to be associated with exposure to fog oil included focal hemorrhage in 3 of 10 rats in the 1.5 mg/l exposure group and multifocal granulomatous pneumonia in 1 of 10 rats. Other changes which were observed within the lung were considered to be incidental and most frequently included peribronchial and perivascular lymphocytic infiltrates.

Multiple foci of macrophage accumulation were present in the cortical and medullary sinusoids of the peribronchial lymph nodes of exposed rats but not in controls. Lymphoid hyperplasia was also observed in the cortex of the peribronchial lymph nodes of many of the exposed rats. The incidence and the severity of these changes were fog oil concentration-related.

At necropsy, the mandibular lymph nodes of several of the control and exposed rats were described as slightly enlarged and red. Microscopically, these lymph nodes had varying degrees of lymphoid hyperplasia and congestions of the cortical and medullary sinusoids. Although the incidence of these findings was slightly greater in exposed rats than in the control rats, the relationship to exposure is not clear. Only mandibular lymph nodes described as abnormal at necropsy were examined histopathologically; therefore, this tissue was not examined from all rats in the study.

No treatment-related changes were present in other tissues examined in this study. A few incidental lesions such as chronic myocarditis, tubular regeneration in the kidney and seminiferous tubular atrophy in the testes were occasionally observed. These changes were considered to be within normal limits for male rats of this age and strain.

3.2.5 Pulmonary Edema

The effects of fog oil treatment on lavage fluid protein content showed a significant main effect due to concentration ($p = 0.0206$). Williams test could not find a trend. It should be noted that this parameter did show significant effects in the 4-week study and that a less conservative subtest (t subtest of least square mean values) showed a significant ($p = 0.009$) increase in lavage fluid protein content at the 1.5 mg/l dose. Increased lavage fluid protein is considered to be indicative of edema, however, subchronic exposure may result in tissue repair or adaptation.

3.2.6 Xenobiotic Metabolism

The effects of fog oil on zoxazolamine-induced paralysis time indicated significantly decreased ($p < 0.0001$) mean paralysis times at the 3 concentrations. Although variances of paralysis times did increase with the mean at each dose, no strong evidence of heteroscedasticity was detectable with Bartlett's test (1937). A two-sided Williams test showed a significantly decreased response even at the 0.5 mg/l level. This suggests that both the 1.5 and 0.5 mg/l fog oil concentrations induced the cytochrome P-448 system.

To further substantiate this conclusion, livers from fog oil-treated and control animals were tested for aryl hydrocarbon hydroxylase (AHH) activity, also an indicator of cytochrome P-448 activity. In addition, since the 4-week exposure studies showed marginally significant effects of fog oil on sodium pentobarbital-induced sleeping time which reflect effects on the cytochrome P-450 system. Cytochrome P-450 levels in livers from fog oil treated animals were also assessed in the subchronic study. However, significant exposure effects were seen. Since Bartlett's test indicated unusual variability for the AHH parameter, a logarithmic transformation was performed prior to statistical analysis. A multivariate ANOVA of liver weight, cytochrome P-450, and the log of AHH activity indicated a strong overall effect of fog oil concentration on the joint response ($p = 0.0001$, Wilks' Criterion). From examination of the univariate ANOVA's, most of this effect stemmed from the increase in AHH activity ($p = 0.0001$) rather than effects on liver weight ($p = 0.20$) or cytochrome P-450 levels (0.33). Williams test showed a significant difference between AHH activity at 0.5 mg/l and control. We conclude from this data that the 90-day fog oil exposure to both 0.5 and 1.5 mg/l induces the cytochrome P-448, but not the cytochrome P-450 system.

3.2.7 Body Weight Change

The effect of fog oil concentration on the difference between beginning and ending body weights for all animals showed a strong influence ($p = 0.0001$) of concentration on these differences. Animals exposed only to air gained 181 g over the course of the exposure. The 0.5 mg/l and 1.5 mg/l groups gained 160 and 113 g, respectively. The two-sided Williams test showed the difference between 0.5 mg/l, 1.5 mg/l and control means were significant. Table 3 summarizes the results of the subchronic (13 week) study.

TABLE 3. SUMMARY OF SUBCHRONIC (13 WEEK) STUDY

Parameter	Effect
Body Wt.	↓ at 1.5 mg/l.
Lung Wt-Dry	↑ at 0.5 and 1.5 mg/l.
Edema	↑ at 1.5 mg/l.
Zoxazolamine-induced Paralysis Time	↓ at 0.5 and 1.5 mg/l.
Aryl hydrocarbon hydroxylase (AHH)	↑ at 0.5 and 1.5 mg/l.
cyt. P-450	No effect.
Immunology	No effects.
Histopathology	↑ AM and lymphoid hyperplasia at 0.5 and 1.5 mg/l.
Clinical Chemistry	↓ triglycerides at 1.5 mg/l
Pulmonary Physiology	No effects.

Adult rats were exposed to fog oil for 4 or 13 weeks at concentrations of 0.5 and 1.5 mg/l. A significant increase in wet lung weights along with the increase in lavage fluid protein suggests the presence of edema in the lungs of animals exposed to 1.5 mg/l fog oil for 4 wk. The increase in dry lung weights after 4 wk may be explained by the hypercellularity observed in these same animals. The increase in total cell number in the high dose animals appears to be caused, in part, by a significant increase in polymorphonuclear leukocytes. These phagocytic cells are known to play an important role in the inflammatory responses of the lung. Additionally, post-mortem analysis of these animals revealed the presence of multifocal pneumonitis in rats exposed to 1.5 mg/l fog oil for 4 wk and major infiltration of alveolar macrophages and lymphoid hyperplasia after 13 wk exposure. Cell totals and differential counts were not performed after the 13 wk subchronic exposure. However, lung weights in the exposed groups were again significantly increased from air controls, and lavage fluid protein was markedly increased in the high exposure animals after subchronic exposure.

Lung volumes, lung compliance and ventilation distribution were not affected by fog oil exposure; however, end-expiratory volume increased, indicating an inflammatory response. We suspect that the increase in EEV allows these animals to ventilate the lung more efficiently with each breath, preventing a decrease in diffusing capacity, and maintaining gas exchange homeostasis. Additionally, we observed an effect of fog-oil on liver xenobiotic metabolism. It appears that fog-oil induces the cytochrome P-448 system but not the cytochrome P-450 system. However, this may be the result of a combined inhalation and ingestion dose and further studies are needed to clarify this finding.

In summary, acute (4 wk) and subchronic (13 wk) inhalation exposure to 1.5 mg/l fog oil appears to cause an inflammatory response in the lungs of adult male rats. However, pulmonary function and gas exchange are not compromised. There are no significant systemic effects at these concentrations aside from an effect on xenobiotic metabolism.